REMARKS

Reconsideration of the application, as amended, is respectfully requested.

Claims 2 and 6 have been amended to eliminate an informality as suggested by the Examiner.

US 5 607 712 (Bourne) discloses a process for improving the firmness of vegetables which are frozen before canning comprising the steps of blanching a vegetable under two different conditions, freezing the blanched vegetable, storing the frozen vegetable, and further successive steps relating to canning the vegetable.

The description indicates that the vegetable can be "frozen quickly" (see column 4 (line 14) and that the frozen vegetable is "usually stored ... at or below 0°F" (see column 4 (lines 21-23)).

Vegetables which are frozen in the conventional manner (i.e. quick-frozen) show, after being cooked from their frozen state, a mushy and poor in-mouth texture (see the present examples). The undersigned has been informed that this is thought to be due to the formation of ice crystals damaging the vegetable cell walls (see page 363 of Fennema et al, enclosed, which the undersigned has enlarged—the undersigned is attempting to obtain clearer copies of Fennema et al. and will furnish them as soon as they are obtained). Fennema et al. is "Low Temperature Preservation of Foods and Living Matter," Marcel Dekker, NY 1973, pp352-385. In particular "several studies with light and electron microscopes have provided support for the supposition that extracellular ice crystals separate and rupture cell walls by accumulation of ice in the middle lamellae" (see page 363 of Fennema et al.) See instant Example 1 and Figures 4-7.

The inventors have devised a process for the production of a frozen vegetable or part which includes the step of under-cooling said vegetable or part thereof to a core temperature of less than or equal to -5°C. The present specification explains that this step "ensures that enough

heat has been removed from the material to allow rapid and uniform ice formation in the freezing step (iii) and thereby provide a significant reduction in extracellular ice formation" (see

page 9 (lines 2-6)). The instant specification further discloses: "It has been shown that merely reducing the core to -1 or -2°C without further under-cooling is not sufficient for the rapid initiation of freezing needed for the desired reduction in extracellular ice..." (see page 9 (lines 8-12).

The Office points to no teaching by US 5 607 712 (Bourne) of undercooling to a core temperature of less than or equal to -5°C and thus applicants submit that the subject matter of claim 1 is inventive over US 5 607 712 (Bourne).

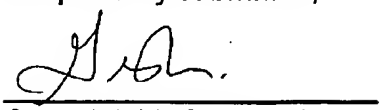
US 3 136 642 (Frane et al), discloses a process for improving the thawing consistency of fruit and vegetable salad for withstanding "the stresses of a quick-freezing and thawing operation" (see column 2 (line 1)). Examples 1 and 2 of Frane et al also disclose that the freezing process is quick–freezing by direct immersion of the subject vegetable pieces in liquid dichlorodiflurомethane (boiling point of -21°F) and liquid octofluorocylobutane (boiling point of -20°F) respectively. The Office again points to no teaching of undercooling to a core temperature of less than or equal to -5°C.

As to Bengtsson, et al., the Office again points to no teaching of undercooling to a core temperature of less than or equal to -5oC. Upon review of the file, applicants have been unable to identify a reference which is listed on Forms PTO-1449 or PTO-892 with that name. Therefore, it would be appreciated if the Office would ensure that Bengtsson et al. is cited of reference and inform applicants of the document type and number.

In view of the foregoing, it is respectfully requested that the application, as amended,

be allowed.

Respectfully submitted,



Gerard J. McGowan, Jr.
Registration No. 29,412
Attorney for Applicant(s)

(201) 894-2297

FOOD SCIENCE

A Series of Monographs

OWEN R. FENNEMA

Volume 1:
FLAVOR RESEARCH: Principles and Techniques
by R. Teranishi, I. Hornstein, P. Issenberg, and E. L. Wick

Volume 2:
PRINCIPLES OF ENZYMOLOGY FOR THE FOOD SCIENCES by John R. Whitaker

Volume 3:
LOW-TEMPERATURE PRESERVATION OF FOODS AND LIVING MATTER
by Owen R. Fennema, William D. Powrie, and Elmer H. Marth

OTHER VOLUMES IN PREPARATION

LOW-TEMPERATURE PRESERVATION OF FOODS AND LIVING MATTER

OWEN R. FENNEMA
Department of Food Science
University of Wisconsin
Madison, Wisconsin

WILLIAM D. POWRIE
Department of Food Science and Agricultural Engineering
University of British Columbia
Vancouver, British Columbia, Canada

ELMER H. MARTH
Department of Food Science
University of Wisconsin
Madison, Wisconsin

MARCEL DEKKER, INC., New York 1973

CHAPTER 7

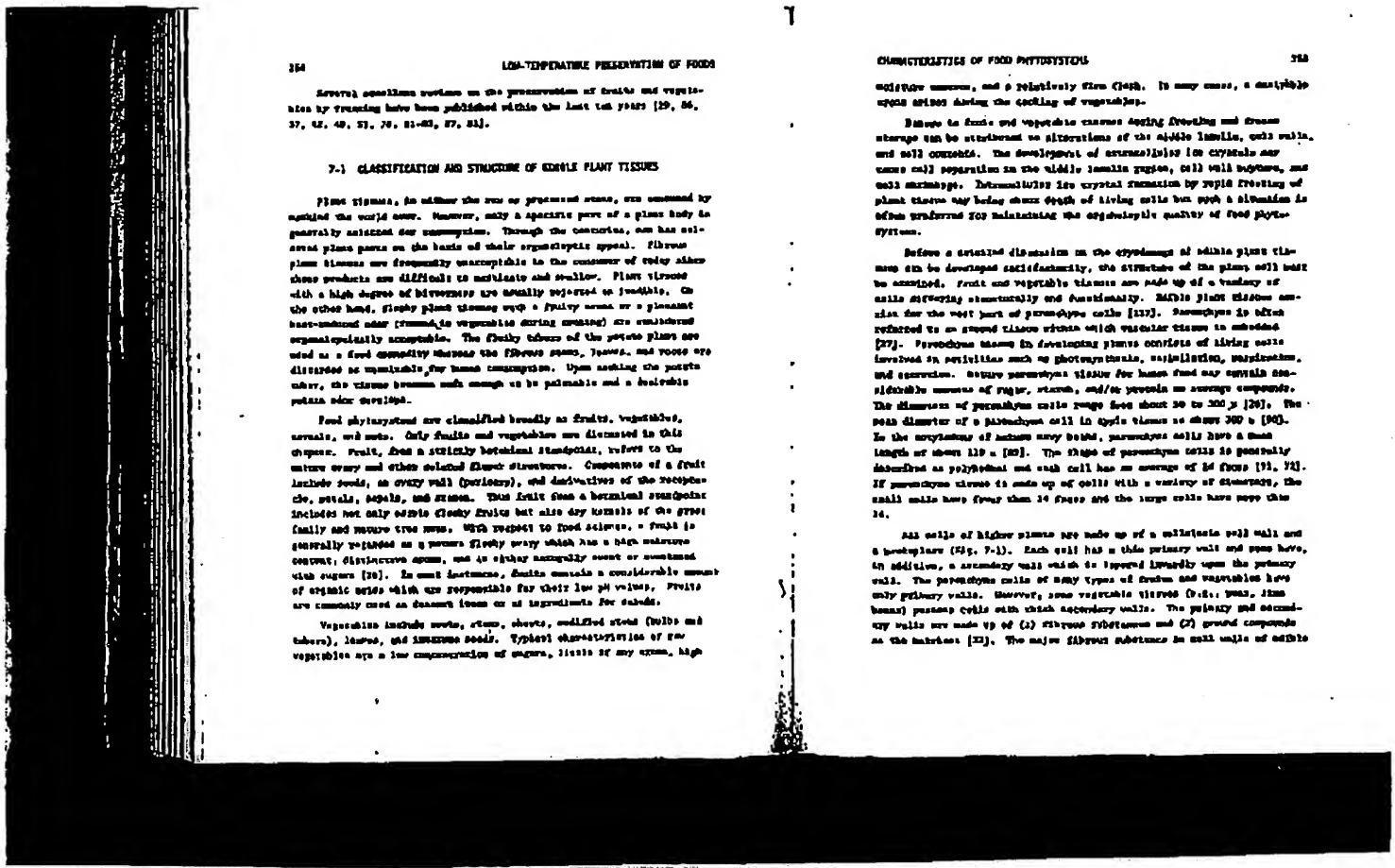
CHARACTERISTICS OF FOOD PHYTOSYSTEMS AND THEIR BEHAVIOR DURING FREEZE-PRESERVATION

Fruits and vegetables or human food (food phytosystems) are in demand throughout the year. Since the predominance of these raw products is seasonal in most parts of the world and since harvested edible plant tissues deteriorate rapidly during storage at ambient temperatures, some method of preservation is essential for long-term storage. Ideally, the native structure and chemical composition of recently harvested fruits and vegetables should be sustained until they are consumed or converted into food products through processing. Such an idealistic procedure would involve inactivation of tissue enzymes, complete retention and stabilization of localized water, maintenance of natural barriers against microbial invasion, and at least partial inactivation of microorganisms.

Enzymes and microorganisms, which are primarily responsible for quality deterioration of harvested plant tissues during storage, can be inactivated by thermal energy but extensive structural and chemical changes of the native tissues are inevitable and frequently undesirable from a quality standpoint. For example, the heat treatment of strawberries alters the delicate flavor of the raw product, reduces the turgidity of the cells, and disrupts the middle lamellae between cells [117].

One approach to maintaining an acceptable quality of some fruits and vegetables for an extended storage period is to reduce the respiration and transpiration rates by lowering the temperature of the product to near the freezing point, and by controlling the O_2, CO_2, and water contents of the microenvironment [19, 20, 207, 208]. Applied in particular have been canned under controlled-atmospheric conditions for periods of up to almost one year with only slight to moderate damage to the eating quality. Storage of fleshy roots and tubers such as carrots and potatoes can be prolonged for many months without significant quality deterioration simply by decreasing the temperature to about 7° or lower. These commodities have relatively low rates of respiration and have natural barrier cells which form a protective coating resistant to microbial attack. However, the chemical composition and quality of many fruits and vegetables are changed even when low nonfreezing temperatures and low levels of oxygen are maintained. Peas, corn, green beans, and asparagus undergo rapid losses of sugars after harvesting because the high rate of respiration cannot be depressed sufficiently by lowering the temperature or controlling the O_2 and CO_2 in the microenvironment. For prolonged storage of food phytosystems, a preservation method such as freezing is essential.

Once perishable plant tissues are frozen and stored under proper conditions, the natural color, flavor, and nutrients are well-retained for several years. However, deviations of some food phytosystems, particularly grains, may be changed substantially by ice crystals. Such cryodamage may impair the textural quality of the thawed product because of a reduction of cell turgidity and a loss of cell fluids or drip (exudate). Addition of sugar to plant tissue prior to freezing has been reported to lessen this type of cryodamage. Frozen) characterization of native fruit tissue can be well-retained by freezing and maintaining small intracellular ice crystals through very rapid freezing, low storage temperatures, and rapid thawing.



Several excellent reviews on the preservation of fruits and vegetables by freezing have been published within the last ten years [29, 36, 37, 42, 49, 51, 70, 81-83, 87, 91].

7-1 CLASSIFICATION AND STRUCTURE OF EDIBLE PLANT TISSUES

Plant tissues, in neither the raw or processed state, are consumed by mankind the world over. However, only a specific part of a plant body is generally selected for consumption. Through the centuries, man has selected plant parts on the basis of their organoleptic appeal. Fibrous plant tissues are frequently unacceptable to the consumer of today since these products are difficult to masticate and swallow. Plant tissues with a high degree of hitherness are usually rejected as inedible. On the other hand, fleshy plant tissues such a fruity aroma or a pleasant heat-induced odor (steamed to vegetables during cooking) are considered organoleptically acceptable. The fleshy tubers of the potato plant are used as a food commodity whereas the fibrous stems, leaves, and roots are discarded as unsuitable for human consumption. Upon cooking the potato tuber, the tissue becomes soft enough to be palatable and a desirable potato odor develops.

Food phytosystems are classified broadly as fruits, vegetables, cereals, and nuts. Only fruits and vegetables are discussed in this chapter. Fruit, from a strictly botanical standpoint, refers to the mature ovary and other selected flower structures. Components of a fruit include seeds, an ovary wall (pericarp), and derivatives of the receptacle, petals, sepals, and stamen. Thus fruit from a botanical standpoint includes not only edible fleshy fruits but also dry kernels of the grass family and mature tree nuts. With respect to food science, a fruit is generally regarded as a mature fleshy ovary which has a high moisture content, distinctive aroma, and is either naturally sweet or sweetened with sugars [20]. In most instances, fruits contain a considerable amount of organic acids which are responsible for their low pH values. Fruits are commonly used as dessert items or as ingredients for salads.

Vegetables include roots, stems, sheets, modified stems (bulbs and tubers), leaves, and immature seeds. Typical characteristics of raw vegetables are a low concentration of sugars, little or any aroma, high

moisture content, and a relatively firm flesh. In many cases, a desirable aroma arises during the cooking of vegetables.

Damage to fruits and vegetable tissues during freezing and frozen storage can be attributed to alterations of the middle lamella, cell walls, and cell contents. The development of extracellular ice crystals may cause cell separation in the middle lamella region, cell wall ruptures, and cell shrinkage. Intracellular ice crystal formation by rapid freezing of plant tissue may being about death of living cells but such a situation is often preferred for maintaining the organoleptic quality of food phytosystems.

Before a detailed discussion on the cryodamage of edible plant tissues can be developed satisfactorily, the structure of the plant cell must be examined. Fruit and vegetable tissues are made up of a variety of cells differing elementarily and functionally. Edible plant tissue consists for the most part of parenchyma cells [117]. Parenchyma is often referred to as ground tissue within which vascular tissue is embedded [27]. Parenchyma tissue in developing plants consists of living cells involved in activities such as photosynthesis, assimilation, meristems, and secretion. Mature parenchyma tissue for human food may contain considerable amounts of sugar, starch, and/or pectin as storage compounds. The dimensions of parenchyma cells range from about 30 to 300 μ [26]. The mean diameter of a parenchyma cell in apple tissue is about 200 μ [90]. In the asymptomy of mature navy beans, parenchyma cells have a mean length of about 110 μ [89]. The shape of parenchyma cells is generally described as polyhedral and each cell has an average of 14 faces [71, 72]. If parenchyma tissue is made up of cells with a variety of diameters, the small cells have fewer than 14 faces and the large cells have more than 14.

All cells of higher plants are made up of a middle lamella, cell wall and a protoplasm (Fig. 7-1). Each cell has a thin primary wall and some have, in addition, a secondary wall which is layered inwardly upon the primary wall. The parenchyma cells of many types of fruits and vegetables have only primary walls. However, some vegetable tissues (e.g., peas, lima beans) possess cells with thick secondary walls. The primary and secondary walls are made up of (1) fibrous substances and (2) ground compounds as the matrices [23]. The major fibrous substance in cell walls of edible

Fig. 7-1. Parenchyma cell of a banana fruit. CW, cell wall; V, vacuole; PM, plasmalemma; T, tonoplast; N, nucleus; P, plastid; M, mitochondrion; RER, roughened endoplasmic reticulum; G, Golgi body. (From Esau and Cavelier, Ref. [78b]; courtesy of Academic Press.)

fruits and vegetables is cellulose (β-1, 4-polyglucan). Cellulose molecules are unbranched polymers consisting of 1000 or more glucose residues. The matrix of the cell wall is made up of hemicelluloses, pectic substances, proteins and lipids.

According to Setterfield and Bayley [82], the average composition of primary cell walls on a dry weight basis is: hemicelluloses, 33%, cellulose, 20%; lipids, 7%; pectin substances, 5%; and proteins, 5%. Lignin may also be present in the primary wall of some plant cells. In the secondary walls, cellulose is the major constituent [32]. Water in the cell walls is located largely in the matrices where it is bound to hydrophilic compounds such as pectin and hemicelluloses.

Cavities are frequently observed in cell walls, particularly in secondary walls [27]. A cavity in the secondary wall is called a pit. A depression in the primary wall has been termed a primary-pit field [115]. Pits are generally arranged in pairs, one being opposite to another in an adjacent cell wall (Fig. 7-2). A pit membrane, made up of

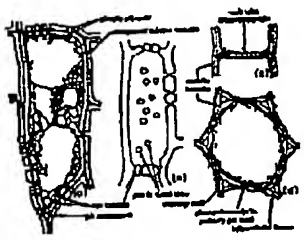
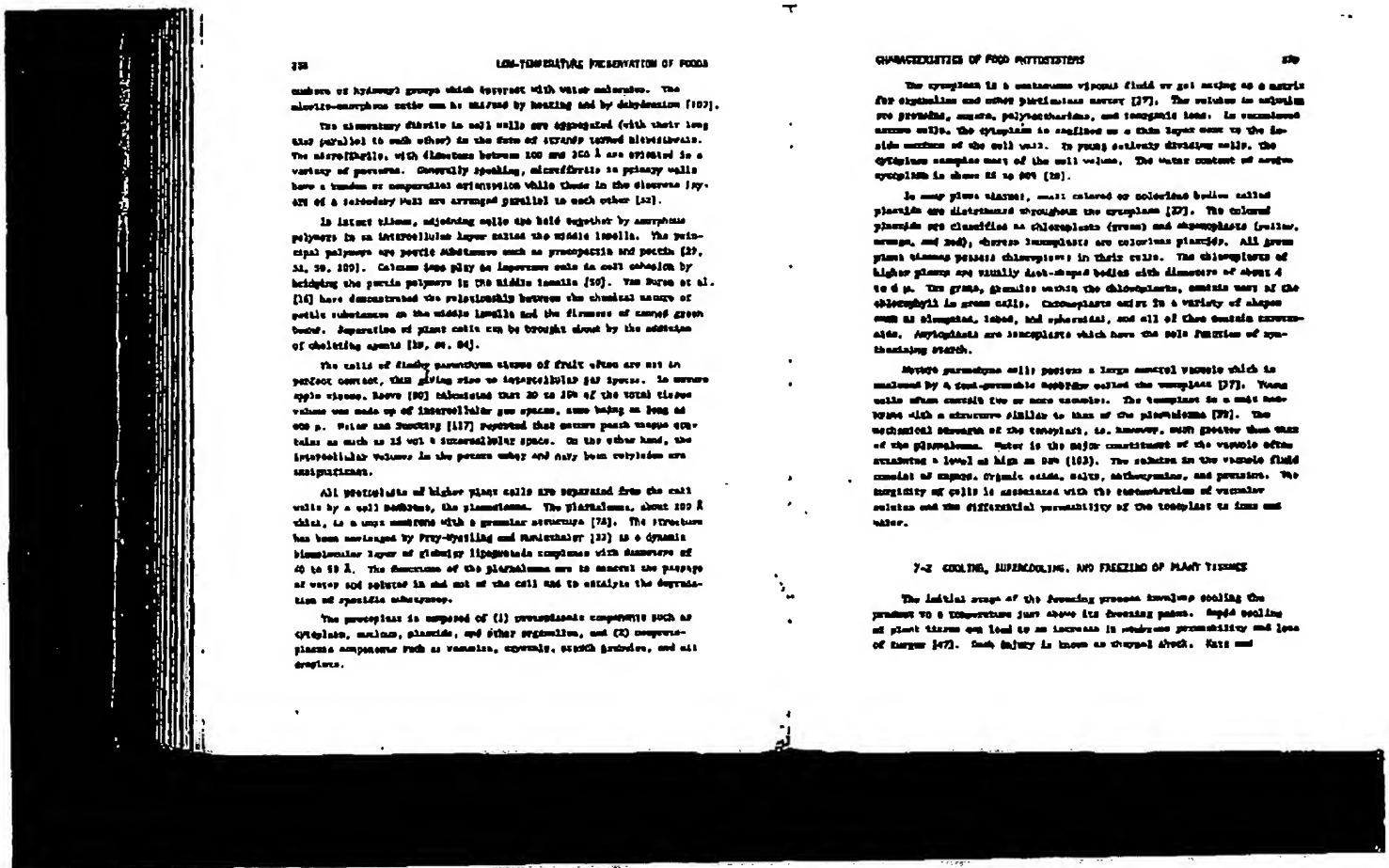


Fig. 7-2. Pits and plasmodesmata in cell walls. (a) and (b), cells with secondary walls; (c) and (d), parenchyma cells without secondary walls. (From Esau, Ref. [27]; courtesy of John Wiley and Sons.)

two thin primary walls with a middle lamella between them, separates paired pits. Several strands (plasmodesmata) extend from the protoplast of one cell into an adjacent cell through the pit membrane. Plasmodesmata are considered to be channels for the flow of nutrients between cells [27].

Cellulose molecules in cell walls of higher plants are not randomly distributed but are oriented longitudinally in the form of bundles. Up to about 100 molecules of cellulose are combined through hydrogen bonding to form ribbonlike elementary fibrils. Cellulose molecules in the elementary fibrils are aligned parallel to their long axes in such a manner that some crystalline regions are formed [27]. These crystalline regions, called micelles, are made up of unit cells each with four glucose residues [32]. Micelles within the primary cell walls have diameters of 35–50 Å, whereas micelles within secondary walls have diameters of 50–60 Å [108]. As much as 50 to 60% of the cellulose in the cell walls of plants is in a crystalline state. The micelles in the elementary fibrils are separated longitudinally by amorphous regions. The micelle regions in cell walls are resistant to enzyme hydrolysis and they absorb very little water [108]. On the other hand, the amorphous regions which contain relatively few cellulose-cellulose hydrogen bonds, possess large



carboxyl groups which interact with water molecules. The microfibrils are held together by amorphous polymers in an intercellular layer called the middle lamella. The principal polymers are pectic substances such as protopectin and pectin [29, 31, 39, 109]. Calcium ions play an important role in cell cohesion by bridging the pectin polymers in the middle lamella [50]. Van Buren et al. [16] have demonstrated the relationship between the chemical nature of pectic substances in the middle lamella and the firmness of canned green beans. Separation of plant cells can be brought about by the addition of chelating agents [29, 84, 94].

The cells of fleshy parenchyma tissues of fruit often are not in perfect contact, thus giving rise to intercellular gas spaces. In mature apple tissue, Bain [90] calculated that 20 to 25% of the total tissue volume was made up of intercellular gas spaces, some being as long as 400 μ. Potter and Dunning [117] reported that mature peach tissue contains as much as 25 vol % intercellular space. On the other hand, the intercellular volumes in the potato tuber and many vegetables are insignificant.

All protoplasts of higher plant cells are separated from the cell walls by a cell membrane, the plasmalemma. The plasmalemma, about 100 Å thick, is a unit membrane with a granular structure [78]. The structure has been envisaged by Frey-Wyssling and Mühlethaler [32] as a dynamic bimolecular layer of globular lipoprotein complexes with diameters of 40 to 50 Å. The functions of the plasmalemma are to control the passage of water and solutes in and out of the cell and to catalyze the degradation of insoluble substrates.

The protoplast is composed of (1) protoplasmic components such as cytoplasm, nucleus, plastids, and other organelles, and (2) nonprotoplasmic components such as vacuoles, enzymes, starch granules, and oil droplets.

The cytoplasm is a continuous viscous fluid or gel acting as a matrix for cytosolic and other particulate matter [37]. The nucleus in eukaryotes prevails, ensures, polysaccharides, and inorganic ions. In unwounded mature cells, the cytoplasm is confined as a thin layer next to the inside surface of the cell wall. In young actively dividing cells, the cytoplasm occupies most of the cell volume. The water content of mature cytoplasm is about 85 to 90% [29].

In many plant tissues, small colored or colorless bodies called plastids are distributed throughout the cytoplasm [37]. The colored plastids are classified as chloroplasts (green) and chromoplasts (yellow, orange, and red); whereas leucoplasts are colorless plastids. All green plant tissues possess chloroplasts in their cells. The chloroplasts of higher plants are usually disk-shaped bodies with diameters of about 4 to 6 μ. The grana, granules within the chloroplasts, contain most of the chlorophyll in green cells. Chromoplasts occur in a variety of shapes such as elongated, lobed, and spheroidal, and all of them contain carotenoids. Amyloplasts are leucoplasts which have the sole function of synthesizing starch.

Mature parenchyma cells possess a large central vacuole which is enclosed by a semi-permeable membrane called the tonoplast [37]. Young cells often contain two or more vacuoles. The tonoplast is a unit membrane with a structure similar to that of the plasmalemma [78]. The mechanical strength of the tonoplast, is, however, much greater than that of the plasmalemma. Water is the major constituent of the vacuole often accounting a level as high as 90% [103]. The solutes in the vacuole fluid consist of sugars, organic acids, salts, anthocyanins, and proteins. The turgidity of cells is associated with the concentration of vacuolar solutes and the differential permeability of the tonoplast to ions and water.

7-2 COOLING, SUPERCOOLING, AND FREEZING OF PLANT TISSUES

The initial stage of the freezing process involves cooling the product to a temperature just above its freezing point. Rapid cooling of plant tissue can lead to an increase in membrane permeability and loss of turgor [47]. Such injury is known as thermal shock. Katz and

Peyphoula [17] found that the cells in the petioles of the plant Coleus lost their turgor upon short exposure to temperatures in the vicinity of 0°. According to these investigators, the electrical conductivity of the tissue increased within the first few hours of exposure to cold temperatures, and then increased suddenly. Such information suggests that the cooling treatment caused an increase in the permeability of the cytoplasmic membrane cell wall to water and ions. On the other hand, Olitz et al. [33] were unable to demonstrate thermal shock in chilled peas which were rapidly cooled to 0°. Thus it is possible but by no means certain that thermal shock of edible plant tissue could contribute to the deterioration of quality that fruits and vegetables undergo during the freezing process.

Supercooling of higher plant tissues is normally confined to a few degrees below the freezing point [58, 74]. Vanderhoyt [64] reported that orange fruit could be supercooled to about -5° and Winkler and Barrett [133] found that some fruits immersed in a coolant could be supercooled to -9°. Under normal commercial freezing conditions, the duration of supercooling in fruit and vegetable tissues is usually brief, i.e., a matter of seconds [12, 83, 85, 103, 118]. However, under special conditions, supercooling of plant tissues can be extended for long periods of time. For example, chlorinated pea shoots can be held in a supercooled state at -6° if the shoots are coated with paraffin to prevent surface nucleation of ice [45].

External water, surrounding metabolically active cells, freezes before the protoplast water of the protoplast [74b]. Even when ice is present on the cell wall surfaces, the protoplast can remain in a supercooled state when freezing temperatures are as low as -12°. Sakai [99, 96] reported that some of the parenchyma cells of the petioles of cabbage leaves at -10° underwent intracellular freezing even when the tissue was seeded with ice. According to Chambers and Hale [15], the living meristem of epidermal cells from scales of a red onion bulb did not freeze at -6° even though ice crystals formed on the surfaces of the cells. When the freezing temperature was dropped to -10°, the protoplasts sometimes suffered damage to the vacuole was not evident immediately after freezing. However, after 1 hr of frozen storage at -20°, the number of ice crystals

localized in the protoplasts and the vacuoles separated into smaller, densely pigmented vesicles with intact membranes surrounding them. Presumably an intact plasmalemma restricts nucleation of intracellular ice in plant tissues at temperatures above -6° [10, 74]. At temperatures below -6°, the critical radius of ice may be smaller than the radius of the pores in the plasmalemma thereby enabling intracellular ice to grow through the intact plasmalemma and promote formation of intracellular ice.

Since intracellular supercooled water has a higher vapor pressure and free energy than external ice at the same temperature, the intracellular water moves through the cell walls and contributes to the growth of extracellular ice crystals [74]. Outward flow of intracellular water will continue as long as the internal water remains unfrozen and has a vapor pressure higher than the external ice. Transport of supercooled intracellular water occurs when plant tissue is frozen slowly and is stored at a relatively high sub-freezing temperature.

The freezing points of edible fruit and vegetable tissues is governed by the concentration of water-soluble solutes (soluble solids) especially sugars, acids, and salts [41]. Sugars are the most predominant soluble solids in food protoplasms. The total soluble solids in fruits and vegetables generally range between 4 and 22% [118]. As shown in Fig. 7-3, the freezing points of fruit and vegetable tissues are directly related to their soluble solids contents.

As the temperature of tissue is decreased below the freezing point, ice and solvent water externally and water-soluble solutes and suspended matter become increasingly more concentrated in the remaining unfrozen aqueous phase. The amount of ice in plant tissues at a given subfreezing temperature is dependent on the initial content of soluble solids. Heidal [92] calculated the ice content of a wide variety of fruits and vegetables at temperatures between -1 and -20° and some data for strawberries, peas, and sweet cherries are shown in Fig. 7-4. In strawberries at -5°, about 80% of the total water is frozen, whereas in sweet cherries, with two and one-half times more soluble solids, only 40% of the total water is frozen. When the temperature is dropped to -20°, 80% or more of the water is frozen in all three plant products.

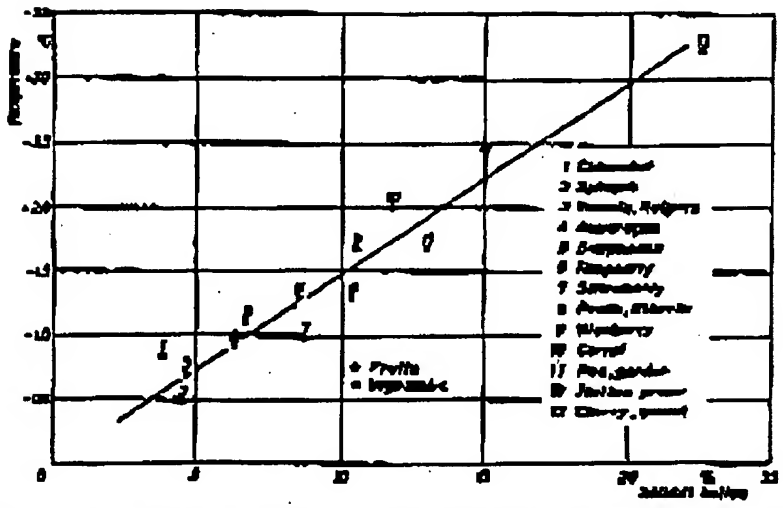


Fig. 7-3. Freezing points of some fruits and vegetables in relation to their soluble solids contents. (From Gutschmidt, Ref. [42]; courtesy of Pergamon Press.)

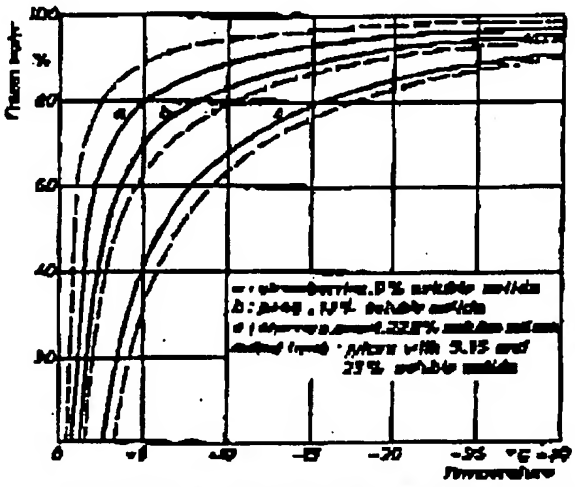


Fig. 7-4. Amount of ice in some food phytosystems at various sub-freezing temperatures (From Gutschmidt, Ref. [42]; courtesy of Pergamon Press.)

7-3 STRUCTURAL AND TEXTURAL DETERIORATION OF PLANT TISSUES DURING FREEZING

7-3.1 Microstructural Alterations

The formation of ice crystals in plant tissues may lead to irreversible damage of the cell walls, middle lamellae, and protoplasts. With such structural damage, textural properties of a thawed food phytosystem may be much inferior to those of the unfrozen tissue. For example, the firmness of apple tissue is reduced considerably by freezing regardless of the rate of ice formation [101]. Several studies have shown that the extent of textural damage (softening of tissue) is related to the degree of tissue disruption [12, 91, 101, 118]. The type and extent of cryodamage to plant tissue is dependent on ice crystal locality and size, and these in turn are governed by the rate of freezing and conditions of frozen storage.

When plant tissue is frozen slowly, ice crystals are located predominantly in extracellular regions [12, 43, 76, 101, 120]. Intercellular spaces are probably the sites where ice crystallization is first initiated [120]. These spaces undoubtedly contain water vapor which condenses on the cell walls as small water droplets and subsequently converts to microscopic ice crystals at freezing temperatures. During exclusive extracellular crystallization, a few large crystals are formed, water is dislocated from the cell and shrinkage of the cell occurs. Sakai [97] found that water withdrawal from plant cells during extracellular freezing took place at temperatures as low as -10°. Such changes are undoubtedly responsible for alterations in the tissue ultrastructure.

With the loss of intracellular water during extracellular freezing, the concentration of inorganic salts increases in the protoplast, perhaps to a level sufficient to precipitate proteins [77]. Levitt [67] postulated that death of plant cells occurs by an irreversible protein-protein interaction involving a sulfhydryl-disulfide interchange.

Several studies with light and electron microscopes have provided support for the supposition that extracellular ice crystals separate and rupture cell walls by accumulation of ice in the middle lamellae [12, 47, 69, 77, 101, 120]. Woodroof [120], using peaches, strawberries, and raspberries, noted microscopically that the cell walls were punctured by

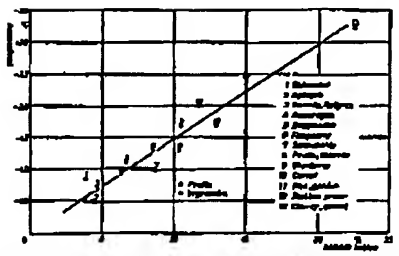


Fig. 7-3. Freezing points of some fruits and vegetables in relation to their soluble solids content. (From Cruess, Ref. [42]; courtesy of Pergamon Press.)

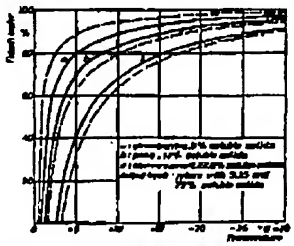


Fig. 7-4. Amount of ice in three plant physiosystems at various sub-freezing temperatures (From Gortner, Ref. [42]; courtesy of Pergamon Press.)

7-3 STRUCTURAL AND TEXTURAL DETERIORATION OF PLANT TISSUES DURING FREEZING

7-3.1 Microstructural Alterations

The formation of ice crystals in plant tissues may lead to irreversible damage of the cell walls, middle lamellae, and protoplasts. With such structural damage, textural properties of a thawed food phytosystem may be much inferior to those of the unfrozen tissue. For example, the firmness of apple tissue is reduced considerably by freezing regardless of the rate of ice formation [181]. Several studies have shown that the extent of structural damage (softening of tissue) is related to the degree of tissue disruption [12, 81, 203, 128]. The type and amount of crystallization in plant tissue is dependent on ice crystal locality and size, and these in turn are governed by the rate of freezing and conditions of frozen storage.

When plant tissue is frozen slowly, ice crystals are located preferentially in extracellular regions [12, 81, 76, 181, 128]. Intercellular spaces are probably the sites where ice crystallization is first initiated [185]. These spaces undoubtedly contain water vapor which condenses on the cell walls as small water droplets and subsequently converts to microscopic ice crystals at freezing temperatures. During exclusive extracellular crystallization, a few, large crystals are formed, water is eliminated from the cell and shrinkage of the cell occurs. Lovel [97] found that water withdrawal from plant cells during extracellular freezing took place at temperatures as low as -20°. Such changes are undoubtedly responsible for alterations in the tissue ultrastructure.

With the loss of intracellular water during extracellular freezing, the concentration of inorganic salts increases in the protoplast, perhaps to a level sufficient to precipitate proteins [77]. Levitt [67] postulated that much of plant cell injury by an irreversible protein-protein interaction involving a sulfhydryl-disulfide interchange.

Several studies with light and electron microscopes have provided support for the supposition that extracellular ice crystals separate and rupture cell walls by accumulation of ice in the middle lamellae [12, 47, 69, 77, 101, 128]. Woodroof [128], using peaches, strawberries, and raspberries, noted histocytologically that the cell walls were fractured by

ice crystals during the freezing process. Dehydration from slowly in air had more than 90% of the cell walls in a fractured state, but with rapid freezing, cell wall damage was only 18%. When Sterling [101] subjected apple tissue to slow freezing conditions, he observed extensive cell separation, cell compression, and rupture of cell walls. In blanched green beans, the fragile cell walls of the inner parenchyma are disrupted by ice crystals even with moderately rapid freezing [12]. With very slow freezing (20 min or longer), the thick-walled cell in the outer parenchyma of the blanched bean pod were often separated from each other but breakage of cell walls was not evident. Freezing damage to blanched bean cells is undesirable even in the thawed product.

Since light microscopy cannot be used to evaluate cryodamage to the fine structure of phytosystems, Mohr and Stein [77] perfected a fixation technique so that the undrawn and frozen parenchyma tissues of tomato fruit could be examined by electron microscopy. Light and electron micrographs of freeze-substituted tomato tissue frozen slowly at rates less than 10° per min revealed enlargement of intercellular spaces, breakage of some cell walls, and expansion of protoplasts to the extent that it occupied only a small fraction of the total volume of each cell. Rupture of the cell walls appeared to occur in the middle lamella region. According to Mohr and Stein [77], endoplasmic reticulum, ribosomes, spherical globules, Golgi bodies, mitochondria, and plastid inclusions were not retained in their original intact status when tomato parenchyma was frozen slowly. Microscopic studies by Penrose and Murray [78] and Reeve and Brown [82] have shown that vascular bundles as well as parenchyma cells are damaged during the slow freezing of asparagus.

When the rate of freezing of plant tissue is greater than about 10° per min, intracellular ice crystals predominate [74, 77]. Electron micrographs of freeze-etched tissue of rapidly frozen tomato parenchyma revealed the presence of very small ice crystals inside and outside the cells as well as in the cell walls [77]. When parenchyma of tomato fruit was frozen at rates greater than 10° per min, Mohr and Stein [77] found that intercellular spaces did not become enlarged, cell walls did not separate or fracture, and the protoplasm remained close to the cell wall since water movement from the cells was restricted. According to Sterling [101], cryodamage to the cell walls of apple tissue frozen in liquid nitrogen was best observed by light microscopy. Similarly, the cell structure of liquid-nitrogen frozen (LNF) green beans appeared to be about the same as that of unfrozen beans [110].

Moderately rapid thawing (1 to 5 min) of rapidly frozen tomato parenchyma causes the least damage to the protoplasm but extensive disruption is still observed [77]. However, the plasmalemma and tonoplast systems of the plastids often remain reasonably intact and sometimes the tonoplasts are not disrupted during the rapid freezing-moderately-rapid thawing region. Maintenance of structural integrity of plant membranes in highly vacuolated cells is important for retention of hydrostatic pressure within the cells, and for the prevention of drip and tissue softening.

Ice crystals in frozen plant tissues usually increase in size during frozen storage and in the early stages of thawing. Such a phenomenon is called recrystallization, and it can have a profound damaging effect on tissue cells and can lead to changes in the microstructure and a loss of tissue firmness. Lusena and Olson [62] observed recrystallization in rapidly frozen epidermal cells of the onion between -4 and -8°. Initially, very small ice crystals were present in the protoplasm, but during the next 2 hr, large intracellular ice crystals were formed. To date, recrystallization within rapidly frozen food phytosystems has been largely ignored by researchers. Studies are needed to determine the influence of temperature of frozen storage, and of temperature of thawing, on the rate of recrystallization in rapidly frozen tissues.

7.3.2 Thermal Alterations

Texture is an important attribute contributing to the overall quality of fruits and vegetables [104]. Texture of food phytosystems is a composite of resistance to bite, feeling on the tongue, juiciness, crispness, and succulence. These sensations are governed by the cellular structure and chemical composition of the tissue. Fresh fruits and vegetables, harvested at proper maturities, are considered as standards or controls for quality comparisons. Some assessing textural damage caused by the freezing process, vegetables are usually cooked as they would be in the home, whereas most fruits are not heat treated.

The influence of freezing rate, as well as frozen storage temperature and time, on the textural qualities of fruits and vegetables has been reviewed recently by several authors [2, 36, 37, 43, 48, 52, 81-83, 117]. General agreement among food scientists has not been reached as to

the effect of freezing rate on the textural quality of vegetables. Goodrich [110] maintained that a short interval between the initial formation of ice crystals and complete freezing was essential for the preservation of textural attributes of vegetables. He recommended a rate of ice formation of 0.1 cm/min. Several investigators have reported that the firmness of blanched asparagus spears was retained to a higher degree by rapid freezing than by slow freezing [53-55, 59, 61, 69]. Lee and Johannessen [61], using a compressimeter and a Kramer shear cell to evaluate the texture of cooked asparagus, concluded that rapid freezing of blanched asparagus spears by immersion in liquid nitrous oxide or sugar syrup at -117.6° was more effective for retaining tissue firmness than slow freezing in moving air at -45.6°. Joslyn and Marsh [53] found less drip from asparagus frozen rapidly in dry ice than from asparagus frozen at -18°.

In a study of blanched peas and green beans frozen at five different rates ranging from rapid (liquid air) to very slow (frozen in an insulated box at either -23.0 or -37.8°), Lee et al. [69] found a significant difference in texture only between peas and beans frozen in liquid air and those frozen at slower rates. All samples were stored for six months at -18° prior to evaluation. The quickly frozen vegetables were much softer than the vegetables frozen at the slower rates. These investigators attributed the softness of the rapidly frozen products to tissue cracking during freezing.

Brown [12] indicated that, if green beans were held in liquid nitrogen after being "completely frozen," the rapid but nonuniform temperature drop caused cracking. To avoid cracking, the vegetable pieces should be size graded and should be removed from the liquid nitrogen within 2 hot after "complete" freezing [12, 118]. Concluding precautions to avoid cracking, Holland and Brown [110] and Brown [12] examined the benefits of rapidly freezing blanched green beans (stored at -29.5°) from the standpoint of texture. These investigators found that cooked beans previously frozen in liquid nitrogen had a firmer texture (measured by Kramer shear press and panel) than beans frozen individually in a sive tray in an air blast at temperatures between -6.7 and -40° or beans frozen in half-pound containers at -28.9°. According to Brown [12], the difference in texture between beans frozen at -6.7° (slow freezing) and those frozen at -40° (blast freezing) was just noticeable. Panel members indicated that cooked beans previously frozen at conventional slow freezing temperatures were

flabby and somewhat rubbery, whereas the texture of the beans frozen in liquid nitrogen was close to that of fresh beans [110]. Prolonged cooking (10 min at about 194°) reduced the textural differences between the rapidly frozen and slowly frozen beans. Recently Szczesniak [41] also reported that green beans frozen in liquid nitrogen, in spite of some cracking, were superior in texture and water-holding ability to those frozen in moving air at -40° or still air at -18° (Fig. 7-5). The beans in this study were stored for only one day.

Lee and Carmean [59] examined frozen lima beans and corn and were unable to detect textural differences between rapidly frozen and slowly frozen samples.

During the slow freezing of fruit tissue, extracellular ice formation can damage the cell walls and middle lamellae to such an extent that the thawed product is much softer than the fresh fruit. Guadagni [42] showed that the panel score for the texture of thawed strawberries previously frozen at -18° (and stored for one day) was much lower than that for the fresh fruit. However, the force required to crush the fresh strawberries was about twice that required for the slowly frozen product (Fig. 7-6). The large amount of drip (about 40% of the total weight) which occurred during thawing of the slowly frozen strawberries was an additional indication of the damage incurred during the freezing process. Bourne [101] reported that the firmness of raw apples as measured by a U. C. Davis Penetrometer was reduced to about one-tenth the original value by freezing the tissue at -10° (slow freezing) followed by immediate thawing.

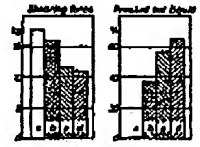


Fig. 7-6. Quality attributes of fresh and frozen green beans. (a) fresh; (b) frozen in liquid nitrogen; (c) frozen quickly at -40°; (d) frozen slowly at -18°. (From Szczesniak, ref. [41]; courtesy of Food Technology.)

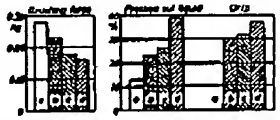


Fig. 7-4. Quality attributes of fresh and frozen strawberries. (a) Fresh; (b) frozen in liquid nitrogen; (c) frozen quickly at −40°; (d) frozen slowly at −18°. (From Guadagni, Ref. [42]; courtesy of Food Technology.)

Since intracellular ice should not damage the cell walls or protoplasm to any appreciable extent, rapid freezing should be advantageous in maintaining the textural quality of fruit tissue. Indeed Guadagni [42] stated that strawberries frozen slowly in still air at −18° were inferior in texture to strawberries frozen in moving air at −40°, or in liquid nitrogen. Moreover the force required to crush the thawed samples was considerably greater for the rapidly frozen strawberries than for those frozen at −18°. According to Bruschweiler [43], delicate fruits such as strawberries and apples do not crush as much during rapid freezing as do blanched vegetables, such as green beans. However, Sterling [101] reported that apple tissue cracked extensively upon freezing in liquid nitrogen, so that the firmness of the thawed product was much less than that of the raw product.

Very little information is available relating the rate of freeze storage to the firmness and textural quality of fruit. Whole frozen and sour pitted cherries in 40% sucrose were held in storage at −6.7°, the firmness as measured by a consistometer increased rapidly during the first five weeks and then leveled off gradually during the next five weeks [43]. Firmness of thawed and drained cherries was found to correlate very well with organoleptic firmness scores of cherries in baked pies. Lee and McCready [34] demonstrated that the increase in firmness of frozen Montmorency cherries was related to the demethoxylation of pectin in the middle lamella of cherries. As the number of free carboxyl groups increase in the pectin molecules, more calcium bridges form between the polymers and this enhances cell cohesion.

7-3.3 Cryoprotective Agents

Although considerable research has been carried out on cryoprotectants for living cells, little information is available on chemicals which effectively protect the textural quality of fruit and vegetable tissue during freezing, storage, and thawing. Sterling [101] found that the texture of apple tissue frozen at −17° can be preserved to some extent in solutions containing either glycerol, ethylene glycol, propylene glycol, polyvinyl pyrrole, or sodium chloride. The firmness of the frozen-thawed apple tissue was maintained better by increasing the concentration of the polyhydroxy cryoprotectants from 5 to 30%. According to Sterling [101], the protective role of the polyhydroxy cryoprotectants involved a restriction of ice crystal size in the intercellular spaces. Sodium chloride was particularly effective in maintaining the firmness of frozen apple tissue, but was ineffective for peaches and strawberries. According to Sterling [101], the main effect on the texture of apple tissue may be attributed to (1) minimization of pectin enzyme activity, (2) migration of salt into cells to increase turgidity and (3) dehydration of the cell walls. The texture of frozen berries also can be improved by adding low methoxyl pectinic acids to the immersion syrup [13, 116].

7-4 CHEMICAL CHANGES IN PLANT TISSUES DURING FREEZING AND FROZEN STORAGE

During frozen storage at temperatures of −7° or lower, microbial growth ceases [78] but chemical changes in food portions may occur even at −18°, the temperature which is commonly used for the commercial storage of frozen fruits and vegetables [36, 37, 48, 51, 81-83, 87]. The major chemical changes which are of importance in quality deterioration of frozen fruits and vegetables are: (1) reactions associated with the development of off-odors and off-flavors, (2) pigment degradation, (3) enzyme browning, and (4) autoxidation of ascorbic acid. Alterations of the pH of plant tissue during freezing and frozen storage may be linked with the rates of these reactions and with deterioration of quality. Satisfactory control of chemical deterioration in frozen preparations can be achieved in most instances by pretreating commodities such as blanching or steaming, and by maintaining the storage temperature at −18° or lower.

7-8.) Reactions Associated with the Development of Off-Odors and Off-Flavors

During frozen storage of unblanched or underblanched vegetables, volatile compounds such as carbonyl compounds and ethanol (anaerobic products of glycolysis) accumulate in the tissue and this accumulation coincides with the development of off-odors [48, 51, 54]. These off-odors persist even after cooking, and may be tentatively described as resembling the odors of:

(1) silage or evaporated grass (artichoke hearts, spinach, asparagus),
(2) alfalfa (peas and green beans), (3) butyric acid (brown beans),
(4) sewage (squash), (5) acrylic acid (broccoli sprouts), and
(6) oxidized oil (lima beans) [51].

Seaforth and Jarvis [48] were the first to report that volatile aldehydes were formed in raw and underblanched plant tissue (green beans) during frozen storage. They found that the acetaldehyde content of green beans decreased and the organoleptic acceptability of the cooked product improved as the duration and temperature of blanching were increased. Later studies showed that acetaldehyde accumulated in other unblanched or underblanched vegetables such as broccoli sprouts, peas, lima beans, and squash during frozen storage [48, 51]. Although acetaldehyde as such does not contribute to the off-odor of frozen vegetables, the aromas present in some freezer food phytosystems correlated with the intensity of off-odor [31, 48, 52]. With some frozen vegetables, investigators [52] have failed to find a correlation between acetaldehyde and off-odors. Saah and Joslyn [14] found that the ethanol content of frozen broccoli shoots was reduced as the degree of off-odor.

The enzyme responsible for the production of acetaldehyde and ethanol in frozen green beans was inactivated at a lower blanching temperature than enzymes involved in the development of off-odors [33]. These researchers also found that, at a storage temperature of -23.3°, formation of acetaldehyde and ethanol in underblanched green beans was inhibited almost completely, yet off-odors developed progressively.

Off-odors in unblanched and underblanched frozen vegetables may be caused by enzymic oxidation of lipids. This lipid degradation is possible when consideration is given to the types and amount of lipids in vegetables. The crude lipid content of vegetables, on a dry weight basis,

ranges between 1.25 and 3.3% [22]. According to Lee and Nawrick [62], the crude lipid component from pea seeds with a chloroform-methanol mixture, consisted of phospholipids, neutral fat, and fatty acids. Substituted (such as linoleic and linolenic acids) of lipoxygenase have been found in peas by Lee and Nawrick [62]. According to Lee and Nagenahalli [63], high peroxide and acid numbers were obtained with lipid from unblanched peas held at -18° for five years. On the other hand, lipids from blanched peas held under the same freezing conditions had peroxide numbers of zero. Furthermore, Lee et al. [64] reported that off-flavors developed in frozen unblanched peas, corn, and okra bread held at -18° for two to four weeks. Moreover, the peroxide and acid numbers of the lipids from these unblanched vegetables increased with frozen storage time. Figure 7-9 illustrates the magnitude of increases in peroxide values for unblanched frozen peas.

According to Lee and Nawrick [64], oxidative rancidity is not the main cause of flavor deterioration in frozen blackeye peas. The highest thiobarbituric acid (TBA) number obtained in raw peas after 404 days of storage at -18° was much below the threshold value for oxidative rancidity. Further, the odor of the frozen raw peas was much different from the rancid odor in blended raw pea slurries with a high TBA number.

Lipoxygenase may play an important role in the oxidation of lipids

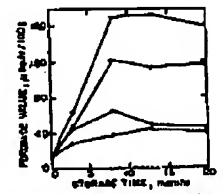
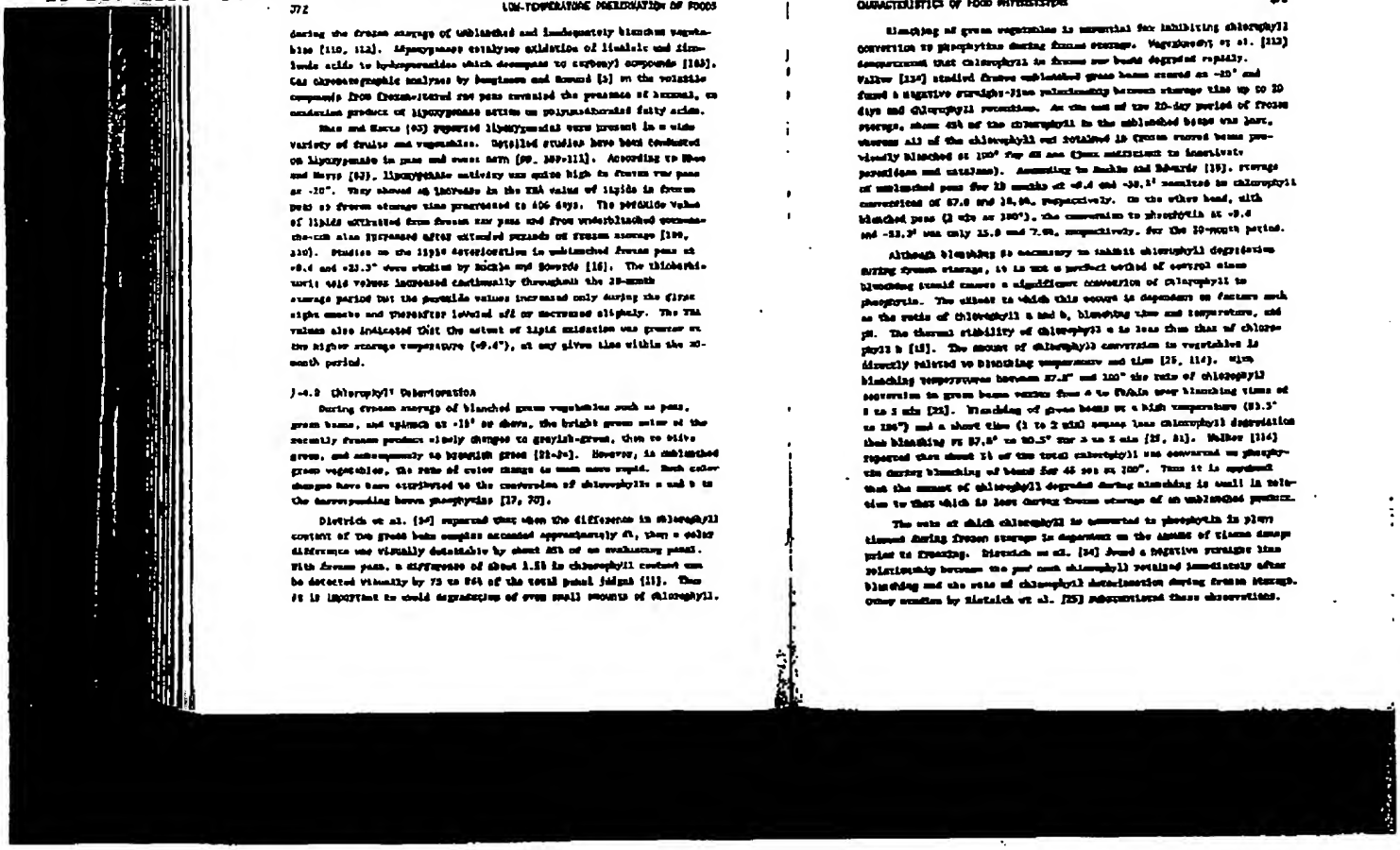


Fig. 7-7. Peroxide values of frozen unblanched peas. ●, stored in oxygen at -8.4°; ■, stored in oxygen at -23.3°; ◆, stored in nitrogen at -8.4°, ▲, stored in nitrogen at -23.3°. (From Ramsis and Edwards; ref. [15]; courtesy of the Society of Chemical Industry, London).



during the frozen storage of unblanched and inadequately blanched vegetables [110, 112]. Lipoxygenase catalyses oxidation of linoleic and linolenic acids to hydroperoxides which decompose to carbonyl compounds [105]. Gas chromatographic analyses by Bengtsson and Hamund [3] on the volatile compounds from freeze-stored raw peas revealed the presence of hexanal, an oxidation product of lipoxygenase action on polyunsaturated fatty acids.

Khan and Harris [93] reported lipoxygenase were present in a wide variety of fruits and vegetables. Detailed studies have been conducted on lipoxygenase in peas and sweet corn [89, 109-111]. According to Khan and Harris [93], lipoxygenase activity was quite high in frozen raw peas at -20°. They showed an increase in the TBA value of lipids in frozen peas as frozen storage time prolonged to 406 days. The peroxide value of lipids extracted from frozen raw peas and from underblanched commercial-cut also increased after extended periods of frozen storage [109, 110]. Studies on the lipid deterioration in unblanched frozen peas at +9.4 and +23.3° were studied by Ritchie and Edwards [16]. The thiobarbituric acid values increased continually throughout the 28-month storage period but the peroxide values increased only during the first eight months and thereafter leveled off or decreased slightly. The TBA values also indicated that the extent of lipid oxidation was greater at the higher storage temperature (+9.4°), at any given time within the 28-month period.

Chlorophyll Deterioration

During frozen storage of blanched green vegetables such as peas, green beans, and spinach at -18° or below, the bright green color of the recently frozen product slowly changes to grayish-green, then to olive green, and subsequently to brownish green [21-24]. However, in unblanched green vegetables, the rate of color change is much more rapid. Such color changes have been attributed to the conversion of chlorophylls a and b to the corresponding brown pheophytins [17, 70].

Dietrich et al. [24] reported that when the difference in chlorophyll content of two green beans amounted approximately 4%, then a color difference was visually detectable by about 50% of an evaluating panel. With frozen peas, a difference of about 1.5% in chlorophyll content can be detected visually by 75 to 84% of the total panel judges [11]. Thus it is important to avoid degradation of even small amounts of chlorophyll.

Characteristics of Food Enzymes

Blanching of green vegetables is essential for inhibiting chlorophyll conversion to pheophytins during frozen storage. Vagnarowski et al. [113] demonstrated that chlorophyll in frozen raw beans degraded rapidly. Walker [114] studied frozen unblanched green beans stored at -20° and found a negative straight-line relationship between storage time up to 20 days and chlorophyll retention. At the end of the 20-day period of frozen storage, about 65% of the chlorophyll in the unblanched beans was lost, whereas all of the chlorophyll was retained in frozen stored beans previously blanched at 100° for 60 sec (long enough to inactivate peroxidase and catalase). Assuming a Hucke and Edwards [16]. storage of unblanched peas for 12 months at +9.4 and -23.3° resulted in chlorophyll conversions of 67.0 and 18.0%, respectively. On the other hand, with blanched peas (2 min at 100°), the conversion to pheophytin at +9.4 and -23.3° was only 15.0 and 7.0%, respectively, for the 28-month period.

Although blanching is necessary to inhibit chlorophyll degradation during frozen storage, it is not a perfect method of control since blanching itself causes a significant conversion of chlorophyll to pheophytin. The extent to which this occurs is dependent on factors such as the ratio of chlorophyll a and b, blanching time and temperature, and pH. The thermal stability of chlorophyll a is less than that of chlorophyll b [15]. The amount of chlorophyll conversion in vegetables is directly related to blanching temperature and time [25, 114]. With blanching temperatures between 87.8° and 100° the rate of chlorophyll conversion in green beans varies from 4 to 9%/min over blanching times of 8 to 2 min [25]. Blanching of green beans at a high temperature (93.3° to 100°) and a short time (1 to 2 min) causes less chlorophyll degradation than blanching at 87.8° to 90.5° for 3 to 5 min [25, 31]. Walker [114] reported that about 1% of the total chlorophyll was converted to pheophytin during blanching of beans for 45 sec at 100°. Thus it is apparent that the amount of chlorophyll degraded during blanching is small in relation to that which is lost during frozen storage of an unblanched product.

The rate at which chlorophyll is converted to pheophytin in plant tissues during frozen storage is dependent on the amount of tissue damage prior to freezing. Dietrich et al. [24] found a negative straight line relationship between the post cook chlorophyll retained immediately after blanching and the rate of chlorophyll deterioration during frozen storage. Comp mention by Dietrich et al. [25] subsequently these observations.

Furthermore, the deterioration rate of chlorophyll at various initial concentrations is doubled as the storage temperature is raised from -12.3 to -3.9° (Fig. 7-4).

Chlorophyll degradation occurs very slowly in frozen beans at -18° and below. The difference in color between control beans (stored at -28.4° or -34.4°) and those stored at -18° first becomes evident to 75% of a panel after an average of 301 days of storage. On the other hand, only about eight days of storage were required for a detectable color difference to develop between control samples at -28.4° and beans stored at -23.9°.

The data in Table 7-1 illustrate that chlorophyll degrades at different rates in frozen peas, green beans, and spinach held at a specific storage temperature. Chlorophyll is converted to pheophytin at a much greater rate in frozen green beans than in frozen peas at any given temperature between -7 and -18°. Neiher [185] reported that during a 12-

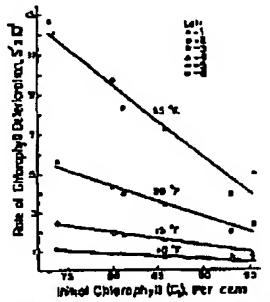


Fig. 7-4. Effect of initial chlorophyll level (C_i) on the rate of chlorophyll deterioration in frozen green beans at various storage temperatures. (From Dietrich et al., Ref. [24]; courtesy of the Institute of Food Technologists.)

TABLE 7-1
Freeze Storage Time (Months) Required for 10% Decrease in Chlorophyll Content of Selected Green Vegetables[a]

Product	Storage temperature, °F		
	-12	-13	-7
Beans	45	12	2.5
Spinach, leaf	30	6	1.5
Spinach, chopped	24	3	0.7
Green beans	20	3	0.7

[a] Olson and Dietrich [24].

month storage period at -18°, the per cent chlorophyll converted to pheophytin was about 45 for brussel sprouts, 65 for green beans, and 6 for peas.

Macerations of plant tissue can accelerate chlorophyll degradation. When spinach leaves are chopped prior to freezing, the rate of chlorophyll conversion to pheophytin is doubled during frozen storage [27].

According to Walker [118], conversion of chlorophyll to pheophytin is not the only cause of color loss in frozen beans. Apparently non-enzymic oxidation of chlorophylls and pheophytins can also occur after 12 months of storage at -18°. Since a marked increase in the peroxide value of lipid in frozen blanched beans occurred after 12 months at -18°, and this coincided with simultaneous oxidation of chlorophylls and pheophytins, it is likely that oxidation of these pigments involves free radicals formed during peroxidation of lipids. The blanching time at 100° can have a profound influence on the rate of chlorophyll oxidation of beans. Walker [114] found that the loss of chlorophyll by oxidation after 10 days storage at -18° was greatest in unblanched green beans, whereas beans blanched for 45 or 60 sec had no detectable loss of chlorophyll. However, as the blanching time increased above 60 sec, the rate of chlorophyll oxidation in the frozen beans increased after an initial lag period of a few days. The rapid oxidation of chlorophyll in unblanched beans and peas may be explained by the catalytic effects of lipoxygenase and a lipohydroperoxide breakdown factor found in many legumes [25, 114].

7.4.3 Enzyme-Catalyzed Oxidative Browning

When raw apples, peaches, pears, cherries, mushrooms, beets, potatoes, and cauliflower are frozen, stored, and thawed, undesirable brown pigments are formed. This reaction is of major concern since sensory evaluation of 12 different lots of commercially frozen peaches indicated that overall quality was directly related to the amount of browning [40]. The browning of plant tissue is usually caused by enzymatic oxidation of phenolic compounds in the presence of oxygen [51, 48].

In pears and apples, chlorogenic acid and catechins are the major substrates of naturally occurring o-diphenol oxidase (polyphenoloxidase, phenolase, tyrosinase) [48]. In potatoes, tyrosine can be rapidly oxidized in the presence of the naturally occurring o-diphenol oxidase. The optimum pH for o-diphenol oxidase is in the vicinity of 6 [56].

Disruption of native cells by ice crystals can initiate enzymatic browning by facilitating contact between o-diphenol oxidase and its substrates. Browning of frozen plant tissue is usually more severe near the surface because atmospheric oxygen is present at a higher level here than in the internal tissue. However, internal as well as surface browning may take place in some frozen-thawed products such as apple tissue since the numerous intercellular spaces generally contain some oxygen [21, 48].

Brown discoloration in frozen plant tissues can be minimized or prevented by: (1) heat inactivation of the enzyme, (2) addition of browning inhibitors such as sulfur dioxide and ascorbic acid, and (3) exclusion of oxygen. Inactivation of o-diphenol oxidase in pear tissue occurs slowly at temperatures between 50 and 60° and rapidly at temperatures of 80° and above [56]. Inactivation of o-diphenol oxidase in apple slices for pies can be achieved without a serious loss of quality by simply blanching prior to freezing [56].

Since blanching of plant tissue may reduce the concentration of desirable odoriferous compounds and perhaps promote development of undesirable cooked odors, discoloration in frozen fruit to be used for dessert purposes should be controlled by treatment with a browning inhibitor. Sulfurous acid and its salts are useful for inhibiting the activity of o-diphenol oxidase in frozen and thawed fruits and vegetables. According to Ponting [56], about 10 ppm of SO_2 is sufficient to inactivate o-diphenol oxidase in apples. For many fruits and vegetables,

penetration of sulfurous acid just below the surface of tissue is sufficient since inhibition of surface browning is of primary concern. However, with apple tissue, complete penetration to the center is essential to control internal browning. Frozen apple slices can be protected against enzymic browning by dipping them in a solution containing 1000–1500 ppm SO_2 for 6 to 8 hr prior to freezing [28]. Frozen slices treated as above contain about 100 ppm SO_2.

Ascorbic acid has been used commercially for many years as an additive to sugar syrup of frozen cut fruit for restricting surface browning [3, 8, 106]. Concentrations of 150 to 250 mg of ascorbic acid per pound of finished product are usually adequate for this purpose. Guadagni et al. [40] reported that the extent and intensity of browning of sliced frozen-thawed peaches were inversely related to ascorbic acid content. In their study, subjective measurement of browning consisted of counting the number of peach slices which had brown discoloration, whereas an objective evaluation of browning was made by obtaining Hunter R_d (reflectance) and b (degree of yellowness) values. It is of importance to note that peach slices submerged first in ascorbic acid-containing syrup and then exposed to air at −6.7°, exhibited brown discoloration within a few weeks. Peach slices that remained completely submerged in syrup at the same temperature did not change in color during a 44-day storage period. Coverage of the slices with sugar syrup (30–31%) apparently restricted oxygen migration into the peach tissue.

Several studies have demonstrated the influence of oxygen content in the head space on the rate of browning. According to Guadagni et al. [41], red sour cherries discolored when they were exposed to air in the head space during frozen storage for 50 days at −6.7°. These cherries were packed in a 60% sucrose syrup which did not contain ascorbic acid. The rate of browning in these frozen cherries was much lower when packed in hermetically-sealed tin cans rather than in metal-end, paperboard-body containers. However if the storage temperature was maintained at −18° or lower, the type of container was not an important factor in the prevention of browning. In another study, Guadagni and Nimmo [39] studied frozen peaches packed in sugar syrup containing ascorbic acid and showed that a vacuum of 15 in. Hg in the headspace of sealed cans prevented discoloration during a storage period of six weeks at −6.7°.

At low concentrations, ascorbic acid does not inhibit the activity of o-diphenol oxidase but rather functions as a reducing agent to keep the oxidizable polyphenols in a reduced state [7, 22]. At high concentrations, the ascorbic acid maintains the polyphenols in the reduced state until o-diphenol oxidase undergoes "reversible inactivation" [24]. In tissues with an active o-diphenol oxidase system, ascorbic acid is gradually oxidized and eventually the polyphenolic compounds are oxidized. Thus it is not surprising that under adverse conditions of frozen storage, ascorbic acid is gradually lost and discoloration of tissue occurs.

Further discussion of methods of inhibiting enzymic browning in fruits and vegetables is presented in Chapter 11.

7-4.A Oxidation of Ascorbic Acid

In the presence of dissolved oxygen, ascorbic acid in aqueous solution is oxidized to dehydroascorbic acid (DHA) which is in turn oxidized irreversibly to 2,3-diketogulonic acid and its degradation products [3]. The rate of ascorbic acid oxidation is directly proportional to the square root of the copper concentration and inversely proportional to the square of the hydrogen ion concentration in acid solutions [25]. Ascorbate oxidase exists naturally in many plant tissues and if it is not inactivated, it will catalyze oxidation of ascorbic acid during the freezing process [3].

If fruits and vegetables are frozen in hermetically sealed metal containers with very little headspace oxygen, no appreciable amount of ascorbic acid will be oxidized. On the other hand, if fruits and vegetables are packaged in composite containers with waxed paper walls and metal ends or in plastic-coated paper board boxes, the oxygen permeability is sufficient to cause substantial deterioration of ascorbic acid during frozen storage.

In addition to the factors cited above, the rate of ascorbic acid oxidation depends on the temperature of frozen storage and pH of the fruits and vegetables. As shown in Fig. 7-8, a high subfreezing temperature of -6.7° results in nearly complete oxidation of ascorbic acid in blanched green peas during a period of three and one-half [23]. In the same product stored at several commercial temperatures of -18°, only about 10% of the total ascorbic acid was oxidized over a one-year period. Similar losses of ascorbic acid occur in beans and spinach.

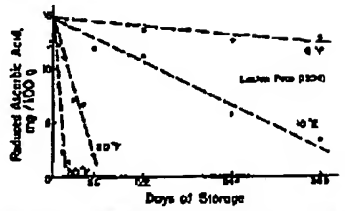


Fig. 7-8. Loss of reduced ascorbic acid in frozen peas at various storage temperatures. (From Ellerbrok et al., Ref. [23]; courtesy of the Institute of Food Technologists.)

As the pH is lowered, the stability of ascorbic acid in food generally improves. For example, the pH of pyrophosphate is much lower than that of green vegetables and as a consequence, ascorbic acid in strawberries at -20° is stable for an 18-mo period [22]. When strawberries are stored at a temperature of -12°, about 50% of the original ascorbic acid is oxidized in about 140 days, but this loss is much lower than that in most green vegetables stored under similar conditions.

In some instances, the rate of oxidation of ascorbic acid in frozen systems either accelerates or declines [23] with elapsed. The circumstances and causes are discussed in Sec. 6-6.

7-4.B Changes in pH

During the freezing of plant tissue, inorganic and organic salts are concentrated in the unfrozen phase. As the freezing temperature decreases, salts may precipitate out of the unfrozen phase at their eutectic points and as a consequence the pH of the unfrozen phase may change. van den Berg [6] and van den Berg and Bean [9] studied pH changes that occur during the freezing of various phosphate buffers. Some of their data are reviewed in Chap. 6 (Sec. 6-3.2). Fruits and vegetables also undergo changes in pH during freezing. van den Berg [7, 8] studied peas stored at -10° and observed that the pH dropped from 6.7 to about 6.0 during the first three days, increased to 7.0 dur-

ing the next two to three weeks and then dropped to 6.4, where it remained to the end of the 75-day storage period. When green beans and cauliflower were frozen to -10°, their pH values dropped to about 6 and 4.8, respectively, during the first 10 days of storage. After a few weeks, pH values increased to about 6. Storage of beans and cauliflower at -18° did not alter the above-mentioned pattern of pH change during storage. van den Berg [7] suggested that the first pH decrease during frozen storage of vegetables was caused by precipitation of alkaline calcium, magnesium, and sodium phosphates. Precipitation of acid potassium phosphate, and sodium and potassium citrate were considered to be responsible for subsequent increases in pH. So far, no studies have shown a relationship between pH change in food phytosystems and quality deterioration during frozen storage. However, it may be speculated that even with a small change in pH, enzyme activity would be altered and cell membranes would be damaged.

REFERENCES

[1] Aylward, F. and D. R. Haisman, Advan. Food Res., 17, 1 (1969).
[2] Bauernfeind, J. C., Advan. Food Res., 4, 359 (1953).
[3] Bauernfeind, J. C. and D. M. Pinkert, Advan. Food Res., 18, 219 (1970).
[4] Bedford, C. L. and M. A. Joslyn, Ind. Eng. Chem., 4, 751 (1939).
[5] Bengtsson, B. and I. Bosund, Food Technol., 18, 773 (1964).
[6] Berg, L. van den, Arch. Biochem. Biophys., 84, 305 (1959).
[7] Berg, L. van den, Food Technol., 15, 434 (1961).
[8] Berg, L. van den, Food Sci., 29, 540 (1964).
[9] Berg, L. van den and D. Rose, Arch. Biochem. Biophys., 81, 319 (1959).
[10] Blair, J. S., Advan. Food Res., 20, 393 (1968).
[11] Boggs, M. M., W. C. Dietrich, M-D. Nutting, R. L. Olson, F. E. Lindquist, G. S. Bohart, H. J. Neumann, and H. J. Morris, Food Technol., 14, 181 (1960).
[12] Brown, H. D., J. Sci. Food Agr., 18, 77 (1967).

[13] Buck, R. P., C. L. Baker and H. W. Wessels, Food Ind., 16, 190 (1944).
[14] Buck, P. A. and M. A. Joslyn, J. Agr. Food Chem., 1, 309 (1953).
[15] Buckle, K. A. and R. A. Edwards, J. Sci. Food Agr., 21, 307 (1970).
[16] Bauer, J. F., ..., J. C. Moyer, D. E. Wilson, W. B. Robinson, and D. B. Hand, Food Technol., 14, 233 (1960).
[17] Campbell, H., Food Res., 2, 55 (1937).
[18] Chambers, T. and H. P. Hale, Proc. Roy. Soc., Ser. B, 110, 336 (1932).
[19] Cormack, R. G. H., Can. J. Bot., 37, 1327 (1959).
[20] Crafts, A. S., H. B. Currier, and C. R. Stocking, Water in the Physiology of Plants, Chronica Botanica Comp., Waltham, Mass., 1949.
[21] David, J. J. and M. A. Joslyn, Food Res., 18, 390 (1953).
[22] Dietrich, W. C., M. M. Boggs, M-D. Nutting, and N. E. Weinstein, Food Technol., 14, 522 (1960).
[23] Dietrich, W. C., F. E. Lindquist, J. C. Miers, G. S. Bohart, H. J. Neumann, and W. F. Talburt, Food Technol., 11, 109 (1957).
[24] Dietrich, W. C., M-D. Nutting, R. L. Olson, F. E. Lindquist, M. M. Boggs, G. S. Bohart, H. J. Neumann, and H. J. Morris, Food Technol., 13, 136 (1959).
[25] Dietrich, W. C., R. L. Olson, M-D. Nutting, H. J. Neumann, and M. M. Boggs, Food Technol., 13, 258 (1959).
[26] Duckworth, R. B., Fruit and Vegetables, Pergamon, Oxford, 1966.
[27] Esau, K., Plant Anatomy, Wiley, New York, 1953.
[28] Fasolin, F. G., C. H. Mallory, and J. R. McConnell, Food Technol., 3, 173 (1949).
[29] Fennema, O. and W. D. Powrie, Advan. Food Res., 13, 219 (1964).
[30] Fidler, J. C., in Low Temperature Biology of Foodstuffs (J. Hawthorn and E. J. Rolfe, eds.), Pergamon, Oxford, 1968, pp. 171-183.
[31] Frey-Wyssling, A., Die pflanzliche Zellwand, Springer, Berlin, 1959.
[32] Frey-Wyssling, A. and K. Mühlethaler, Ultrastructural Plant Cytology, Elsevier, New York, 1965.

[33] Fuischi, T. and J. J. David, Food Technol., 17, 1323 (1963).
[34] Cox, M. and R. M. McCready, Food Res., 22, 300 (1957).
[35] Gilles, O. G., F. A. Back, A. C. Rice, and V. O. Fortin, J. Food Sci., 27, 300 (1962).
[36] Guadagni, D. G., in Low Temperature Biology of Foodstuffs (J. Hawthorn and E. J. Rolfe, eds.), Pergamon, Oxford, 1968, pp. 399-412.
[37] Guadagni, D. G., in Quality and Stability of Frozen Foods (W. B. Van Arsdel, M. J. Copley, and R. L. Olson, eds.), Wiley, New York, 1969, pp. 85-116.
[38] Guadagni, D. G. and F. W. Kelly, Food Technol., 12, 645 (1958).
[39] Guadagni, D. G. and C. C. Nimmo, Food Technol., 11, 43 (1957).
[40] Guadagni, D. G., C. C. Nimmo and E. F. Jansen, Food Technol., 11, 33 (1957).
[41] Guadagni, D. G., C. C. Nimmo and E. F. Jansen, Food Technol., 12, 36 (1958).
[42] Gutschmidt, J., in Low Temperature Biology of Foodstuffs (J. Hawthorn and E. J. Rolfe, eds.), Pergamon, Oxford, 1968, pp. 299-318.
[43] Guyer, R. M., R. D. Lovejoy, and L. M. Beacham, J. Assoc. Offic. Agr. Chem., 34, 821 (1951).
[44] Hendershott, C. H., Proc. Am. Soc. Hort. Sci., 80, 247 (1962).
[45] Hulle, G., van, O. [illegible], and D. D. Perrin, J. Food Sci., 30, 601 (1966).
[46] Hulme, A. C., Advan. Food Res., 8, 297 (1958).
[47] Idle, D. B. and M. A. Hudson, in Low Temperature Biology of Foodstuffs (J. Hawthorn and E. J. Rolfe, eds.), Pergamon, Oxford, 1968, pp. 155-175.
[48] Joslyn, M. A., Advan. Enzymol., 9, 613 (1949).
[49] Joslyn, M. A., Econ. Bot., 1, 447 (1953).
[50] Joslyn, M. A., Advan. Food Res., 11, 1 (1962).
[51] Joslyn, M. A., in Cryobiology (H. T. Meryman, ed.), Academic, New York, 1966, pp. 565-607.
[52] Joslyn, M. A. and G. L. Bedford, Ind. Eng. Chem., 32, 702 (1940).
[53] Joslyn, M. A. and D. D. Tilmer, Quick Frozen Foods, 4(12), 14 (1942).

[54] Joslyn, M. A. and G. L. Marsh, Fruit Prod. J., 11, 327 (1932).
[55] Joslyn, M. A. and G. L. Marsh, Fruit Prod. J., 13, 308 (1934).
[56] Joslyn, M. A. and J. Miller, Food Research, 14, 325 (1949).
[57] Kato, K. and L. Reinhold, Israel J. Bot., 13, 106 (1964).
[58] Lee, F. A., Advan. Food Res., 8, 63 (1958).
[59] Lee, F. A. and W. A. Gortner, Refrig. Eng., 57, 148 (1949).
[60] Lee, F. A., W. A. Gortner, and J. Whitcombe, Ind. Eng. Chem., 38, 341 (1946).
[61] Lee, F. A. and O. A. Johannessen, Food Technol., 5, 163 (1951).
[62] Lee, F. A. and L. R. Mattick, J. Food Sci., 26, 273 (1961).
[63] Lee, F. A. and A. C. Wagenknecht, Food Res., 16, 239 (1951).
[64] Lee, F. A., A. C. Wagenknecht and J. C. Hening, Food Res., 20, 289 (1955).
[65] Le Saint, A.-M., Rev. Gen. Bot., 13, 814 (1956).
[66] Levitt, J., in Cryobiology (H. T. Meryman, ed.), Academic, New York, 1966, pp. 495-563.
[67] Levitt, J., Cryobiology, 2, 343 (1966).
[68] Luyet, B. J. and M. C. Gibbs, Biodynamica, 1(25), 1 (1937).
[69] MacArthur, M., Sci. Agr., 20, 186 (1940).
[70] MacKinney, G. and C. A. Weast, Ind. Eng. Chem., 32, 392 (1940).
[71] Matsko, R. D., Am. J. Bot., 33, 58 (1946).
[72] Matsko, R. D., Am. J. Bot., 34, 182 (1947).
[73] Maney, A. H. and R. B. Harvey, Sci. Agr., 21, 510 (1933).
[74] Mazur, P., in Cryobiology (H. T. Meryman, ed.), Academic, New York, 1966, pp. 214-315.
[75] Mitchener, H. D., P. A. Thompson, and E. C. Dietrich, Food Technol., 14, 29 (1960).
[76a] Modlibowska, I., in Low Temperature Biology of Foodstuffs (J. Hawthorn and E. J. Rolfe, eds.), Pergamon, Oxford, 1968, pp. 125-133.
[76b] Mohr, W. P. and E. C. Cocking, J. Ultrastructure Res., 21, 171 (1968).
[77] Mohr, W. P. and M. Stein, Cryobiology, 6, 15 (1969).
[78] Moor, H., K. Mühlethaler, H. Waldner, and A. Frey-Wyssling, J. Biophys. Biochem. Cytol., 10, 1 (1961).

[79] [illegible], T. N., in Low Temperature Biology of Foodstuffs (J. Hawthorn and E. J. Rolfe, eds.), Pergamon, Oxford, 1968, pp. [illegible].

[80] [illegible], A. D., Science, 112, [illegible] (1951).

[81] Olson, R. L., in Low Temperature Biology of Foodstuffs (J. Hawthorn and E. J. Rolfe, eds.), Pergamon, Oxford, 1968, pp. [illegible]-377.

[82] Olson, R. L. and W. C. Dietrich, in The Freezing Preservation of Foods (D. K. Tressler, W. B. Van Arsdel, and M. J. Copley, eds.), Vol. 2, Avi Publishing Co., Westport, Conn., 1968, pp. 83-106

[83] Olson, R. L. and W. C. Dietrich, in Quality and Stability of Frozen Foods (W. B. Van Arsdel, M. J. Copley, and R. L. Olson, eds.), Wiley, New York, 1969, pp. 117-141.

[84] [illegible], C. J. and P. F. Sharp, Food Res., 4, 299 (1939).

[85] Phillips, W. R., Refrig. Eng., 53, 401 (1947).

[86] Ponting, J. D., in Food Enzymes (H. W. Schultz, ed.), Avi Publishing Co., Westport, Conn., 1960, pp. 105-124.

[87] [illegible], J. D., B. Feinberg, and P. P. Boyle, in The Freezing Preservation of Foods (D. K. Tressler, W. B. Van Arsdel, and M. J. Copley, eds.), Avi Publishing Co., Westport, Conn., 1968, pp. 107-133.

[88] Ponting, J. D. and M. A. Joslyn, Arch. Biochem., 19, 47 (1948).

[89] Powrie, W. D., H. V. Adams and I. J. Pflug, Agron. J., 52, 163 (1960).

[90] Reeve, R. M., Food Res., 18, 604 (1953).

[91] Reeve, R. M. and M. S. Brown, Cryobiology, 3, 214 (1966).

[92] Riedel, L., Kältetechnik, 2, 195 (1950).

[93] [illegible], R. B. and T. M. [illegible], J. Food Sci., 31, 664 (1966).

[94] [illegible], K. S. and G. M. [illegible], J. Food Sci., 31, 675 (1966).

[95] Sakai, A., Low Temp. Sci., Ser. B, 19, 1 (1961).

[96] Sakai, A., Nature, 193, 89 (1962).

[97] Sakai, A., Plant Physiol., 40, 882 (1965).

[98] [illegible], E. and S. T. [illegible], Ann. Rev. Plant Physiol., 13, 33 (1962).

[99] Siddiqi, A. M. and A. L. Tappel, Arch. Biochem. Biophys., 60, 91 (1956).

[100] Sterling, C., Recent Adven. Food Sci., 2, 259 (1962).

[101] Sterling, C., J. Food Sci., 33, 577 (1968).

[102] Sterling, C. and F. J. Shimazu, J. Food Sci., 26, 479 (1961).

[103] Stocking, C. R., in Encyclopedia of Plant Physiology (W. Ruhland, ed.), Vol. 3, Springer-Verlag, Berlin, 1956, pp. 15-27.

[104] Szczesniak, A. S. and [illegible], Food Technol., 19, 74 (1965).

[105] Tappel, A. L., in Lipids and Their Oxidation (H. W. Schultz, E. A. Day, and R. O. Sinnhuber, eds.), Avi Publishing Co., Westport, Conn., 1962, pp. 122-138.

[106] Tressler, D. K. and C. W. DuBois, Food Ind., 16, 701 (1944).

[107] Ulrich, R., Ann. Rev. Plant Physiol., 9, 385 (1958).

[108] Varner, J. E., Ann. Rev. Plant Physiol., 12, 245 (1961).

[109] Wagenknecht, A. C., Food Res., 24, 539 (1959).

[110] Wagenknecht, A. C. and F. A. Lee, Food Res., 21, 605 (1956).

[111] Wagenknecht, A. C. and F. A. Lee, Food Res., 23, 25 (1958).

[112] Wagenknecht, A. C., F. A. Lee, and P. P. Boyle, Food Res., 17, 343 (1952).

[113] Walker, G. C., J. Food Sci., 29, 383 (1964).

[114] Walker, G. C., J. Food Sci., 29, 389 (1964).

[115] Wardrop, A. B., Botan. Rev., 28, 241 (1962).

[116] [illegible], J. B., R. H. Baer and F. D. Rodgers, Quick Frozen Foods, 14, No. 1, 60 (1951).

[117] Weier, T. E. and C. R. Stocking, Advan. Food Res., 3, 297 (1949).

[118] Whiteman, T. M., Freezing Points of Fruits, Vegetables and Florist Stocks, U. S. Dept. Agr. Mktg. Res. Rept., 1957, p. 196.

[119] Wolford, E. R. and M. J. Brown, Food Technol., 19, 188 (1965).

[120] Woodroof, J. G., "Microscopic Studies of Frozen Fruits and Vegetables", Georgia Inst. Technol., Eng. Expt. Sta. Bull. No. 201, 1938.

[121] Woolrich, W. R. and A. M. Bartlett, Mech. Eng., 64, 447 (1942).